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Mutagenic Activity of Irradiated C₂H₄/NO_x
Mixtures in the Presence of Diethylhydroxylamine

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THE MUTAGENIC ACTIVITY OF IRRADIATED C_2H_4/NO_x MIXTURES IN THE PRESENCE OF DIETHYLHYDROXYLAMINE

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Abstract—Mixtures of ethylene and oxides of nitrogen were irradiated in the absence and presence of diethylhydroxylamine. As previously reported, the presence of diethylhydroxylamine inhibited the photo-oxidation of the hydrocarbon and nitric oxide and the onset of ozone formation. Once the diethylhydroxylamine completely reacted, the ozone rose more rapidly, but to a lower level than in the absence of diethylhydroxylamine. Peroxyacetyl nitrate was also produced with the addition of diethylhydroxylamine. The reaction mixtures were tested for total mutagenic activity by gas phase exposure to *Salmonella typhimurium* strain TA100. A significantly greater mutagenic activity was observed in the irradiated ethylene/diethylhydroxylamine/oxides of nitrogen mixture relative to the irradiated ethylene/oxides of nitrogen mixture. At most, 30% of the observed response could be accounted for by known reaction products.

Key word index: DEHA, diethylhydroxylamine, photochemical smog, mutagenicity, Ames test, ethylene.

INTRODUCTION

Over the past several years a number of papers have appeared in the literature suggesting the use of diethylhydroxylamine (DEHA) as a chemical inhibitor of photochemical smog (Jayanty *et al.*, 1974; Olszyna and Hecklen, 1976; Schaal *et al.*, 1978; Stockburger *et al.*, 1976). The use of DEHA as a smog inhibitor has been reviewed by Hecklen (1981). Although DEHA (at ~ 0.25 ppm) has been shown by Schaal *et al.* (1978) and Cupitt and Corse (1979) to inhibit hydrocarbon oxidation effectively at roughly atmospheric hydrocarbon/oxides of nitrogen (HC/NO_x) levels, the results of the work of Pitts *et al.* (1977) is in disagreement with this finding. Although Hecklen (1981) has presented possible explanations for the discrepancy, there is still some controversy as to what concentrations of DEHA will effectively inhibit photochemical smog formation.

An important concern, that must be thoroughly addressed before a chemical such as DEHA is added to the atmosphere, is whether DEHA itself or the reaction products from the photooxidation of DEHA are toxic or mutagenic. Hecklen (1981) has reported the results of extensive inhalation studies and mutagenicity testing of DEHA and its known oxidation products (e.g. nitroethane). Diethylhydroxylamine and its photo-oxidation products that were tested were found to be 'not very toxic'. However, there is the possibility that minor unknown oxidation products that are very toxic

or genotoxic are present (Cupitt, 1981), but this has not been addressed experimentally.

We have recently demonstrated that a smog chamber operated in a flow mode can be used to conduct an assay of the mutagenic activity of the complete air mass in an irradiated HC/NO_x mixture, using an adapted version (Shepson *et al.*, 1985a) of the Ames test (McCann *et al.*, 1975). The products obtained in a propylene/oxides of nitrogen (C_3H_6/NO_x) photo-oxidation (Kleindienst *et al.*, 1985a) showed a mutagenic activity (for TA100) at least four times greater than that calculated from the mutagenic activities of the individual known products. In this paper, we report the results of a 20-h exposure of *Salmonella typhimurium* strain TA100 to the reactant and product distribution corresponding to a ~ 2.7-h irradiation of 1 ppm ethylene ($C_2H_4/0.25$ ppm $NO_x/0.24$ ppm DEHA in humidified air).

EXPERIMENTAL

These experiments were conducted in a 22.7-m³ Teflon smog chamber, which is irradiated with a combination of sunlamps and blacklights. Most of the experimental apparatus and analytical techniques have been described previously (Shepson *et al.*, 1985a). A schematic of the experimental apparatus is presented in Fig. 1. Diethylhydroxylamine (Aldrich, 97%; major impurity, water) was added to the chamber by bubbling N_2 through an impinger bottle contain-

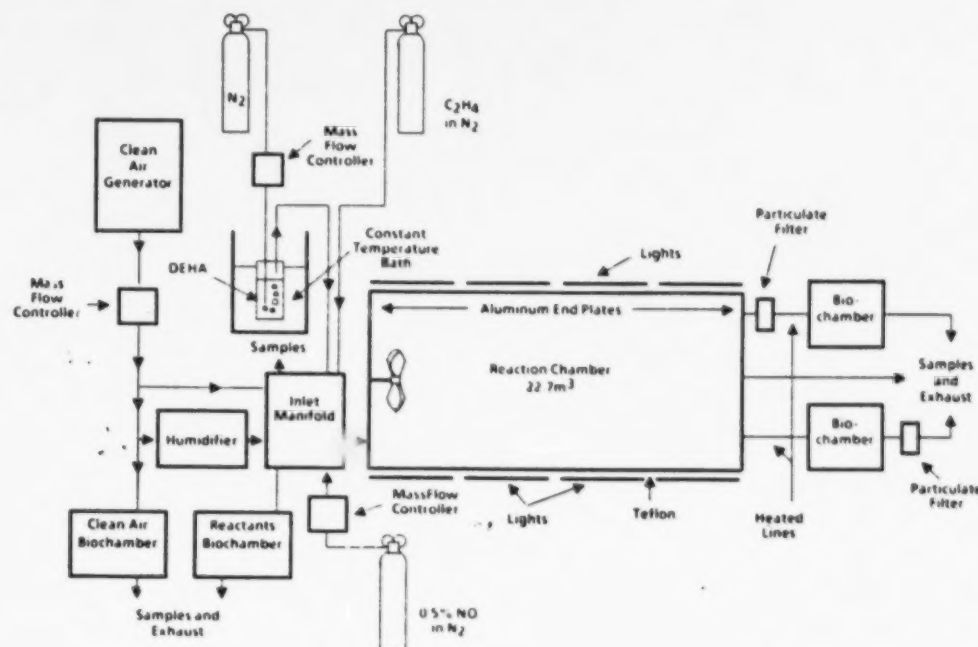


Fig. 1. Experimental schematic.

ing DEHA cooled to 0°C. The C_2H_4 and NO were metered through mass flow controllers and mixed with humidified clean air in a 150- ℓ stainless steel inlet manifold. The total input flow was controlled with a mass flow controller at 140 ℓ min $^{-1}$. This is the highest flow that is experimentally attainable in the dynamic mode, and it yields a 2.7-h reaction chamber residence time. For the static mode irradiations, the reactant gases were allowed to flow into the chamber until they reached the desired levels. The reactant flows were then shut off, and the total flow of diluent air was set at 14 ℓ min $^{-1}$ to allow for an equivalent sampling rate.

The sampling and analytical techniques for the measurement of NO, NO_2 , O_3 , peroxyacetyl nitrate (PAN), formaldehyde (HCHO), acetaldehyde (CH_3CHO), relative humidity, and the physical parameters were conducted as previously described (Shepson *et al.*, 1985a). For the C_2H_4 /DEHA/ NO_2 static mode experiment, NO_2 was measured with a Bendix NO_2 monitor which has a carbon-based converter. For all other experiments, a Monitor Labs NO_2 monitor was used, which has a molybdenum-based converter. (The Bendix instrument responds to nitroethane, while the Monitor Labs instrument does not.) Ethylene was measured using a Varian 1400 gas chromatograph (FID) containing a 3.2-mm $\times$ 2-m stainless steel column packed with 60/80 Carbowise G. Ethylene standards were prepared by diluting 100 μ l of pure C_2H_4 (MG Scientific) with 100 ℓ of pure air in a Teflon bag. Diethylhydroxylamine was measured by bubbling (at 1 ℓ min $^{-1}$) 15 ℓ of air from the chamber (or inlet manifold) through glass impinger bottles containing 2 ml methanol cooled to 0°C. Ten-microliter aliquots of the methanol sample were then injected into an HP5985 GC/MS, containing a 3.2-mm $\times$ 2-m glass column packed with 10% SP-1000 on 80/100 Supelcoport. The column was maintained at 70°C for 1 min, followed by heating to 150°C at 20°C min $^{-1}$. The GC/MS was operated in the selected ion monitoring (SIM) mode, using characteristic DEHA masses at $m/e = 74$ and 89. The GC/MS was calibrated using standard samples of DEHA in methanol. Since DEHA

concentrations were found to decay in methanol at $\sim 1\%$ h $^{-1}$, all standards and samples were run within 1 h after their preparation. The sampling efficiency of this technique, determined to be 99%, was obtained by using two bubbler samples in series. The DEHA wall loss rate in the chamber was found to be 4.3% h $^{-1}$.

For the *S. typhimurium* exposures, the reaction chamber was operated in a dynamic mode. The experiments were conducted by bringing the reactant (in the inlet manifold) and product concentrations to steady state values at a reactor residence time of 2.7 h. The reactor effluent was then continuously transferred (at 14 ℓ min $^{-1}$) through heated ($\sim 50^\circ$ C) Teflon lines (50 cm $\times$ 0.8 cm I.D.) to two 190- ℓ biochambers. (The residence time of the effluent in the heated lines was approximately 0.1 s.) Two separate C_2H_4 /DEHA/ NO_2 exposures were conducted at identical reaction conditions. In the first C_2H_4 /DEHA/ NO_2 exposure, both effluent biochambers contained plates with and without S9 mix. In this experiment, one of the biochamber lines was filtered (using a glass fiber filter) to remove any mutagens present in the aerosol phase. In the second experiment, in which no filtering was done, one effluent biochamber contained plates without S9 mix, and the other contained plates with S9 mix. In this way, we could determine if, in the first set of experiments, any mutagenic metabolites produced in the S9 plates had volatilized and then deposited into the plates without S9, yielding a response in the non-activated media. In addition, as shown in Fig. 1, a clean air biochamber and a reactants biochamber were employed as controls to check for mutagenic activity in the clean air and reactant gases, respectively. All exposure chambers were maintained at 25°C during the experiment.

The exposures were conducted by loading the biochambers with 25 covered glass Petri dishes containing *S. typhimurium* strain TA100 with metabolic activation (S9 mix) and 25 plates without metabolic activation. The plates were prepared by adding 0.1 ml of *S. typhimurium* culture (containing $\sim 10^8$ bacteria) to 3 ml of an agar overlay at 45°C (with or without 0.5 ml S9 mix). This mixture was then poured onto ~ 4 ml of

plate agar in a glass Petri plate. The *S. typhimurium* tester strain TA100 was provided by Dr Bruce Ames (University of California, Berkeley, CA, U.S.A.). Colony counting was done with an Artec 880 automatic colony counter using previously published guidelines (Claxton *et al.*, 1984). The test procedures used were those of Ames *et al.* (1975), except for the following modifications: (1) glass Petri dishes were used, (2) 45 ml of minimal medium agar per plate was used, (3) minimal histidine at the same final total concentration was placed in the bottom agar rather than the top agar, and (4) 3 ml of overlay agar with $\sim 1 \times 10^8$ bacteria were used. The rat-liver homogenate (S9) fraction was prepared from male Charles River CD-1 rats (Wilmington, MO, U.S.A.) induced with Aroclor 1254 (Ames *et al.*, 1975). Once the biochambers were loaded, they were sealed, and the biochamber concentrations were allowed to achieve steady state values. At this point, the plates were uncovered, effectively starting the 20-h exposures. The exposures were conducted for 20 h, at 25°C, and the total gas volume through each biochamber was 17 m³. At the end of the 20-h period, they were incubated at 37°C for 48 h, and then revertant colonies were counted at the Health Effects Research Laboratory facility.

RESULTS

To define better the effectiveness of DEHA as a smog inhibitor and the extent of reaction of the air masses used for the two dynamic mode exposures, C_2H_4/NO_x and $C_2H_4/DEHA/NO_x$ static mode irradiations were performed. The initial C_2H_4 , NO , and NO_2 concentrations were roughly 1, 0.2, and 0.05 ppm, respectively. The initial DEHA concentration for the $C_2H_4/DEHA/NO_x$ static experiment was 0.24 ppm. This follows the suggestion that the initial DEHA/HCl ratio be ~ 0.25 with respect to the OH reactivity of C_2H_4 (Heicklen, 1981). These two experiments were conducted in $\sim 40\%$ relative humidity air. During

these experiments, the following reactants and products were measured periodically: C_2H_4 , DEHA, NO , NO_2 , O_3 , PAN, HCHO, and CH_3CHO . The concentration-time profiles for these two experiments are presented in Figs 2 and 3.

The C_2H_4/NO_x and $C_2H_4/DEHA/NO_x$ exposures were conducted at nominally the same reactant concentrations. At this residence time, which roughly corresponds to a 2.7-h reaction time in the static experiment, the DEHA has been completely consumed, and significant quantities of its reaction products should be present. The 2.7-h residence time was selected to minimize to the extent possible secondary reactions of DEHA oxidation products. During these exposures, which lasted for 20 h, the same reactant and product concentrations as in the static experiments were periodically measured. The average concentrations measured during the exposures are presented in Table 1.

The mutagenesis results are presented in Table 2 for the two photooxidation systems. For the system containing DEHA, duplicate exposures were performed, and the data for each exposure are listed individually. Spontaneous controls given in the first row of Table 2 measure the natural reversion rate in the absence of added mutagens. Sodium azide (NaN_3) and 2-aminoanthracene were employed as the positive controls. In addition to the regular test plates, survivor plates were also employed during the exposures. These plates were placed in each exposure chamber to check for possible toxicity of the effluent or reactant gases. Depressed levels of survivor colonies with respect to laboratory controls were never observed for these

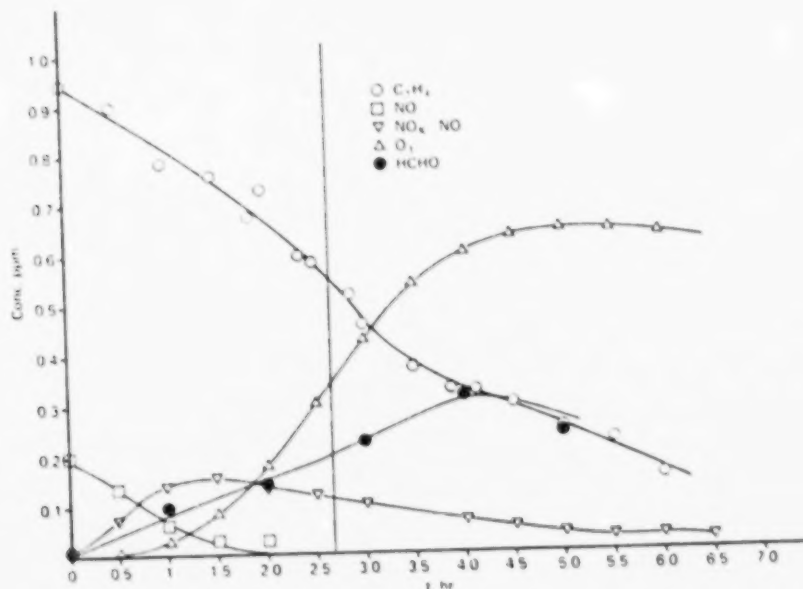


Fig. 2. C_2H_4/NO_x static irradiation reactant and product profiles.

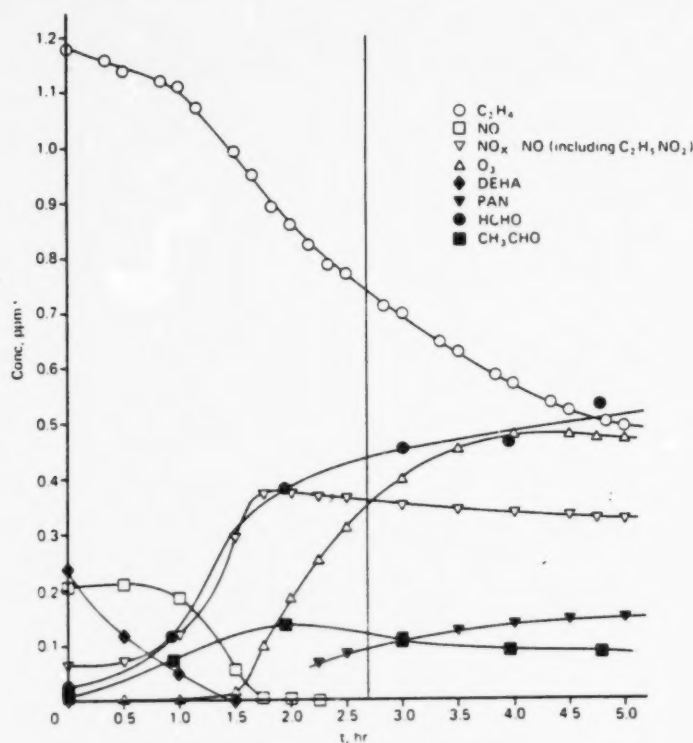


Fig. 3. $C_2H_4/DEHA/NO_x$ static irradiation reactant and product profiles.

experiments. The number of excess (i.e. induced) revertants is calculated by subtracting the number of revertants in the clean air biochamber (which represent the background values) from the number of revertants in the reactant or effluent biochambers. These values (not tabulated) will be used for comparisons.

DISCUSSION

As can be seen in Figs 2 and 3, addition of DEHA at $\sim 20\%$ of the initial C_2H_4 concentration decreases the HC removal rate by about a factor of two, which is somewhat less than indicated by Heicklen (1981) and by Cupitt and Corse (1979). Since OH reaction with DEHA presumably does not yield peroxy-type radicals, the addition of DEHA significantly delays oxidation of NO to NO_2 , as shown in Fig. 3. In addition, it should be noted that the maximum O_3 concentration in the static experiment is ~ 0.17 ppm lower in the presence of 0.24 ppm DEHA initially, for the same nominal HC/ NO_x ratio, 5:1. (Although the NO_x -PAN value is greater than zero at the ozone peak, this excess is probably due to nitroethane and ethyl nitrate and not to NO_2 .) This is in contrast to the findings of Pitts *et al.* (1977) and Cupitt and Corse (1979), both of which indicate that under most con-

ditions addition of DEHA ultimately leads to a higher O_3 maximum than in the absence of DEHA. The work of Cupitt and Corse (1979) did, however, indicate that the amount of O_3 increase (or decrease) is a complex function of the DEHA/HC and HC/ NO_x ratios. The data taken together indicate that a general conclusion cannot be drawn as to whether addition of DEHA increases or decreases O_3 formation. Although it appears that DEHA will inhibit HC photooxidation to some extent as long as it is present, the possibility of toxic/hazardous reaction products being formed must also be carefully considered.

As seen in Table 2, after subtracting the background (i.e. clean air) revertant values, the irradiated $C_2H_4/DEHA/NO_x$ mixture exhibits significantly greater mutagenic activity than does the irradiated C_2H_4/NO_x mixture. This occurs to an extent of roughly 400 excess revertants. The survivor levels in the effluent biochamber were essentially the same as the laboratory survivor level, indicating that there were no apparent toxicity effects in these exposures and that the exposures would likely be within the linear portion of a dose-response curve. Since there is no significant difference in results between the filtered and unfiltered biochambers, we can assume that the mutagens present are gas phase species. However, if particulate mutagens were formed in this system, they might be difficult to

Table 1. Average reactant and product concentrations (ppm*)

Species	C ₂ H ₄ /NO _x exposure		C ₂ H ₄ /DEHA/NO _x exposure†	
	Inlet	Effluent	Inlet	Effluent
C ₂ H ₄	1.16(0.03)	0.618(0.012)	1.15(0.038) 1.10(0.080)	0.641(0.023) 0.559(0.031)
DEHA	—	—	0.239(0.017) 0.239(0.012)	0 0
NO	0.238(0.008)	0	0.256(0.003) 0.268(0.002)	0 0
NO _x	0.278(0.014)	0.120(0.014)	0.286(0.010) 0.283(0.003)	0.193(0.013) 0.272(0.005)‡
O ₃	—	0.467(0.011)	—	0.282(0.004) 0.314(0.012)
HCHO	—	0.327	—	0.306(0.046) 0.220(0.035)
CH ₃ CHO	—	0	—	0.098(0.009) 0.087(0.013)
PAN	—	0	—	0.113(0.005) 0.128(0.003)
Relative humidity		33%		26% 22%

*Standard deviation in parentheses.

†Two sets of numbers refer to duplicate experiments.

‡Includes C₂H₃NO₂.Table 2. Measured mutagenic activity for exposure of TA100 to experimental gas streams in revertants plate⁻¹*

	C ₂ H ₄ /NO _x exposure		C ₂ H ₄ /DEHA/NO _x exposure†	
	w/o S9	w/S9	w/o S9	w/S9
Spontaneous	177(21)	158(15)	208(14) 168(10)	185(11) 177(13)
3 µg NaN ₃ ‡ (-S9)	559	—	591 439	—
0.5 µg 2-aminoanthracene‡ (+S9)	—	890	—	534 392
Clean air biochamber	188(19)	199(24)	240(26) 163(14)	209(37) 175(19)
Reactants biochamber	221(21)	222(22)	215(37) 152(20)	231(38) 174(23)
Filtered effluent biochamber	234(35)	245(23)	660(43)	616(63)
Unfiltered effluent biochamber	242(38)	257(18)	629(58) 498(45)	614(58) 444(51)

*Standard deviation of 25 plates in parentheses.

†Two sets of numbers refer to duplicate exposures.

‡Positive control mutagens

detect since particulates have relatively low deposition velocities and the plate loading tends to be low under these experimental conditions (Shepson *et al.*, 1985b). The gas phase mutagens in the irradiated C₂H₄/DEHA/NO_x mixture appear to be direct acting since the revertant level does not increase with the addition of S9. However, an increase in activity due to metabolic activation might be difficult to detect, since the activity of the S9 enzyme mix continuously decreases following plating, and the exposure occurs over

a 20-h period. The data from the reactants biochamber indicate that DEHA itself is not a mutagen for TA100 at these exposure levels. This is confirmed by standard plate incorporation tests. In light of the uncertainties presented, we will focus the remaining discussion on direct-acting, gas phase mutagenic products.

One method to account for the observed results is to sum the mutagenic contributions from the individual components of the mixture. This can be done to a first approximation by assuming that all gas phase species

Table 3. Maximum plate product concentrations [$\mu\text{mole plate}^{-1}$ from Equation (1)] and mutagenic activities

Species	Mutagenic activity (revertants μmole^{-1})	$\text{C}_2\text{H}_4/\text{NO}_x$		$\text{C}_2\text{H}_4/\text{DEHA}/\text{NO}_x$	
		Plate conc.	Max. revertants	Plate conc.	Max. revertants
C_2H_4	BDL*	0	0	0	0
DEHA	BDL	0	0	0	0
NO	BDL	0	0	0	0
NO_2	BDL	—†	0	—†	0
O_3	BDL	6.4	0	3.9	0
HCHO	12‡	4.5	54	4.2	51
CH_3CHO	BDL	—	0	—	0
PAN	34	0	0	1.5	51
HNO_3	BDL	—	0	—	0
$\text{C}_2\text{H}_5\text{NO}_2$	BDL	—	0	—	0
$\text{C}_2\text{H}_5\text{ONO}_2$	BDL	—	0	—	0
H_2O_2	19	(0.3)§	(6)	(0.3)§	(6)
CH_3ONO_2	BDL	—	0	—	0
		Totals	54 (60)¶		102 (108)¶

*BDL—below detection limit (≤ 2 revertants μmole^{-1}).

†Not determined.

‡Toxic at $> 6 \mu\text{moles plate}^{-1}$.

§Based on estimated upper limit of 25 ppb in gas phase completely depositing into the test plates.

¶Includes the estimated contribution from H_2O_2 .

passing through the biochambers are deposited into and equally distributed among the 50 test plates. Previous work in our laboratories (Shepson *et al.*, 1985a) indicates that this is a fairly good approximation for polar species, such as HCHO. The concentration of each chemical per plate is then calculated using Equation (1)

$$C_p = C_g \cdot F_c \cdot T_e \cdot (1/N_p) \quad (1)$$

where C_p is the concentration of each species in the plates, C_g is the molar gas phase concentration, F_c is the flow rate through the biochambers, T_e is the total exposure time, and N_p is the total number of plates. The values calculated from Equation (1) for each of the products in the two dynamic exposures are presented in Table 3. Also listed in Table 3 are HNO_3 , H_2O_2 , and CH_3ONO_2 , which were not measured but were expected to be present. Of these three, only H_2O_2 was found to be mutagenic. Using a luminol technique (Kok *et al.*, 1978), which apparently suffers from both positive and negative artifacts (Zika and Saltzman, 1982), we have estimated gas phase H_2O_2 concentrations of 10 ppb at a 2.7-h extent of reaction for the $\text{C}_2\text{H}_4/\text{NO}_x$ system. We have, therefore, assigned a 25-ppb upper limit for the gas phase H_2O_2 concentration in both systems. Assuming H_2O_2 is completely and uniformly deposited in all test plates, 0.3 μmoles would be deposited per plate for a 20-h exposure, as given in Table 3.

We have conducted a number of single-component gas phase exposures (e.g. see Kleindienst *et al.*, 1985b) and standard plate incorporation tests for the various

products measured and also for nitroethane and ethylnitrate, which are products of DEHA oxidation (Stockburger *et al.*, 1976). The mutagenic activities of these species, expressed as revertants μmole^{-1} per plate are presented in Table 3. All activities are presented for TA100 without S9 metabolic activation. For the three products that were found to be mutagenic (HCHO, PAN and H_2O_2), their mutagenic activities were obtained by gas phase exposure using the pure chemical. The contribution of each chemical to the total observed mutagenicity of the mixture can be determined by multiplying each chemical's calculated plate concentration by its measured mutagenic activity, as shown in Table 3. These data indicate that the roughly 50 excess revertants observed in the $\text{C}_2\text{H}_4/\text{NO}_x$ exposure can be accounted for by the HCHO, although the uncertainty in the observed total response is of the same order. Although HCHO and PAN can account for a significant fraction ($\sim 30\%$) of the observed response in the $\text{C}_2\text{H}_4/\text{DEHA}/\text{NO}_x$ exposures, there is a missing mutagenic component(s). In addition, recent measurements have indicated that approximately 20% of the PAN present in the exposure chambers dissolves under these conditions and, therefore, in reality PAN probably accounts for about 10 revertants plate^{-1} .

Thus, we consider the possibility that oxidation of DEHA may lead to some unknown product or products (presumably present in small quantities) which may be strong mutagens. It is known that the DEHA oxidation product $\text{C}_2\text{H}_5\text{OH}$ is non-mutagenic. [In fact, Ames *et al.* (1975) suggests its use as a solvent in

the standard plate incorporation test.] Another product found by Hecklen (1981) is N_2O . However, given its extremely high vapor pressure, we suspect that N_2O would not dissolve into the test medium. The ethylnitrite of CH_3CHO , a semistable product, presumably reacts rapidly with O_3 and NO_2 (Hecklen, 1981), and its biochamber concentration at the 2.7-h residence time is probably small. In addition, there are currently no data available regarding its mutagenic activity. Therefore, we are uncertain of the identity of additional mutagens.

Recently, we have begun to study the mutagenic activity of irradiated CH_3CHO/NO_x mixtures. Preliminary results indicate that a mutagenic response is observed in this system even at relatively short extents of reaction. The magnitude of the response indicates that part of the mutagenic activity in the irradiated DEHA system could be attributed to products in the irradiated CH_3CHO/NO_x system. Further experiments with this system are being conducted.

CONCLUSIONS

Although an irradiated mixture of C_2H_4/NO_x yields very little mutagenic activity in this adaptation of the Ames test, irradiated $C_2H_4/DEHA/NO_x$ mixtures yield a significant observed response (~ 400 excess revertants per plate). Increased mutagenic activity has also been seen in other HC/NO_x photooxidation systems (Kleindienst *et al.*, 1985a). In the irradiated C_2H_4/NO_x system, mutagenic activity from the products has been observed. The response is within a factor of two of that observed in this system when normalized to amount of reacted hydrocarbon (C_2H_4 vs DEHA). (Based on our previous work with the irradiated C_2H_4/NO_x system, a large fraction of the observed mutagenic response is apparently due to minor products that we have not identified as yet.) The small response in the C_2H_4/NO_x system can be accounted for based on the mutagenic activity of HCHO. However, the only two known mutagens present (HCHO, PAN) in the irradiated $C_2H_4/NO_x/DEHA$ system cannot account for the larger response observed when DEHA is present. Part of the mutagenic activity appears to be due to photooxidation products of CH_3CHO produced in the DEHA system. The other portion of the mutagenic activity could be due to HCHO produced from C_2H_4 oxidation and minor products from the oxidation of DEHA. As an alternative explanation, the observed response may be caused by some cooperative effect involving more than one oxidation product. Therefore, although we have corroborated the observation that DEHA can effectively inhibit HC photooxidation at certain $HC/NO_x/DEHA$ levels, it appears that more research into the possible mutagenic effects of minor products is warranted.

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